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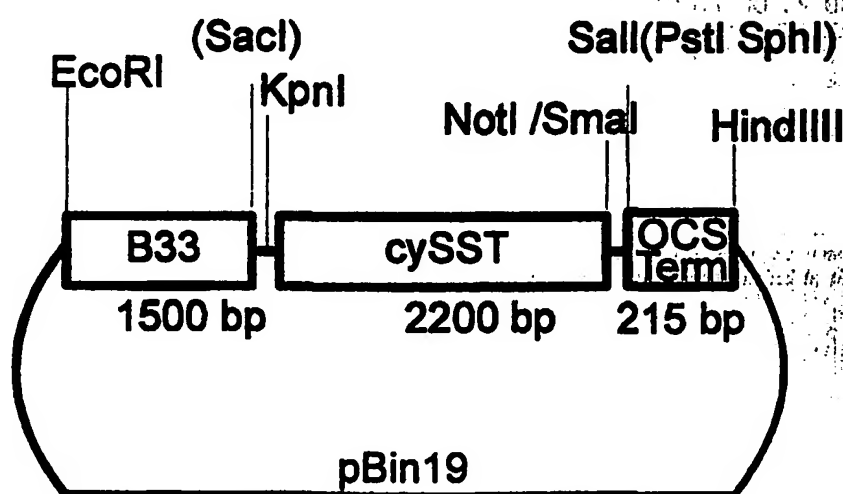
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(54) Title: NUCLEIC ACID MOLECULES FROM ARTICHOKE (*CYNARA SCOLYMUS*) ENCODING ENZYMES HAVING FRUCTOSYL POLYMERASE ACTIVITY



(57) Abstract

Described are nucleic acid molecules encoding enzymes having fructosyl polymerase activity. These enzymes are sucrose dependent sucrose fructosyltransferases (SST) enzymes. Furthermore, vectors and host cells are described containing the nucleic acid molecules, in particular transformed plant cells and plants that can be regenerated from them and that express the described SSTs. Furthermore, methods for the production of short-chain fructosyl polymers using the described hosts and/or the SSTs produced by them are described.

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NUCLEIC ACID MOLECULES FROM ARTICHOKE (CYNARA SCOLYMUS) ENCODING ENZYMES HAVING FRUCTOSYL POLYMERASE ACTIVITY

The present invention relates to nucleic acid molecules encoding sucrose dependent sucrose fructosyltransferases (SST). Furthermore, this invention relates to vectors and hosts containing such nucleic acid molecules, as well as plant cells and plants transformed with the described nucleic acid molecules. Furthermore, methods for the production of transgenic plants are described that synthesize short-chain fructosyl polymers due to the introduction of DNA molecules encoding an SST from artichoke. The present invention also relates to methods for the production of SST for producing short-chain fructosyl polymers in various host organisms as well as to the SST with the help of which short-chain fructosyl polymers can be produced using various methods, for example fermentative or other biotechnological methods.

Water-soluble, linear polymers have many various applications, for example for increasing the viscosity of aqueous systems, as detergents, as suspending agents or for accelerating the sedimentation process and for complexing but also for binding water. Polymers on the basis of saccharides, for example fructosyl polysaccharides, are especially interesting raw materials since they are biodegradable.

Apart from their application as regenerative raw materials for industrial production and processing, fructosyl polymers are also interesting as food additives, for example as artificial sweeteners. Polymers having a low polymerization level are particularly suitable for this purpose.

Up to now only processes for the production of long-chain fructane polysaccharides in plants by expression of enzymes of bacterial origin as well as a process for the production of transgenic plants expressing fructosyltransferases from *Helianthus tuberosus* have been described. Processes for the production of enzymes for producing short-chain fructosyl polymers are not known. In the specification of PCT/USA89/02729 the possibility to produce carbohydrate polymers, in particular

dextrane or polyfructose, in transgenic plants, in particular in the fruits of transgenic plants, is described. For the production of such modified plants the use of levane sucrases from microorganisms, in particular from *Aerobacter levanicum*, *Streptococcus salivarius* and *Bacillus subtilis*, or from dextrane sucrases from *Leuconostoc mesenteroides* are suggested. The production of neither the active enzymes nor of levane or dextrane nor of transgenic plants is described. The specification of PCT/EP93/02110 discloses a process for the production of transgenic plants expressing the lsc gene of levane sucrose from the gram-negative bacterium *Erwinia amylovora*. In the specification of PCT/NL93/00279 the transformation of plants having chimeric genes that contain the sacB gene from *Bacillus subtilis* or the fft gene from *Streptococcus mutans* is described. In the case of the sacB gene a modification in the 5'-untranslated region of the gene is recommended in order to increase the expression level in transgenic plants. The specification of PCT/NL96/00012 discloses DNA sequences encoding the enzymes synthesizing carbohydrate polymers and the production of transgenic plants with the help of these DNA sequences. The disclosed sequences originate from *Helianthus tuberosus*. According to PCT/NL96/00012 the disclosed sequences are not only suitable to modify the fructose profile of, for example, petunia and potato but also of *Helianthus tuberosus* itself. Therefore, the specification of PCT/NL96/00012 describes inter alia transgenic potato plants expressing an SST from *Helianthus tuberosus*. Even though the enzymatic activity of the SST expressed in the transgenic plants could be detected, only a low level of conversion of the substrate sucrose to short-chain fructosyl polymers could be achieved. This may be related to various factors, such as a low affinity of the enzyme to its substrate or a possible inhibition of the enzyme by the produced product.

Therefore, the problem of the present invention is to provide nucleic acid molecules encoding a sucrose dependent sucrose fructosyltransferase (SST) with the help of which it is possible to produce organisms modified by genetic engineering that are able to form short-chain fructosyl polymers.

This problem is solved by providing the embodiments described in the claims.

Therefore, the present invention relates to nucleic acid molecules encoding the proteins having the biological activity of an SST and being selected from the group consisting of

- (a) nucleic acid molecules encoding a protein that comprises the amino acid sequence depicted in SEQ ID No. 2 and SEQ ID No. 4;
- (b) nucleic acid molecules comprising the nucleotide sequence depicted in SEQ ID No. 1 or a corresponding ribonucleotide sequence;
- (c) nucleic acid molecules comprising the nucleotide sequence depicted in SEQ ID No. 3 or a corresponding ribonucleotide sequence;
- (d) nucleic acid molecules hybridizing to the nucleic acid molecules mentioned in (a) or (b) and encoding an SST the amino acid of which is to at least 90 % identical to the amino acid sequence depicted in SEQ ID No. 2; and
- (e) nucleic acid molecules the nucleotide sequence of which deviates from the sequence mentioned in (a), (b) or (c) due to the degeneration of the genetic code.

In the context of the present invention an enzyme having the fructosyl polymerase activity is understood to be a protein that is able to catalyze the linking of β -2,1 glycosidic or β -2,6 glycosidic bonds between fructose units. Hereby, a fructosyl residue to be transferred can originate from sucrose or a fructan polymer. A short-chain fructosyl polymer is understood to be a molecule containing at least two but not more than 100 fructosyl residues that are linked either β -2,1 glycosidically or β -2,6 glycosidically. The fructosyl polymer can carry a glucose residue at its terminal that is linked via the C-1 OH-group of the glucose and the C-2 OH-group of a fructosyl. In this case a molecule of sucrose is contained in the fructosyl polymer.

In a preferred embodiment the nucleic acid sequences of the invention are derived from artichoke.

It was surprisingly found that during the expression of the nucleic acid molecules of the invention large amounts of fructosyl polymers were produced.

In contrast to the potatoes described in the specification of PCT/NL96/00012 a large amount of oligofructan is obtained that is even larger than the cellular content of the substrate sucrose when the nucleic acid molecules of the invention are used.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. The nucleic acid molecules of the invention can be isolated from natural sources, preferably artichoke, or can be synthesized according to known methods.

By means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) to introduce different mutations into the nucleic acid molecules of the invention. As a result proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. By such deletions at the 5'-terminal of the nucleotide sequence it is, for example, possible to identify amino acid sequences that are responsible for the translocation of the enzyme in the plastids (transition peptides). This allows the specific production of enzymes that are due to the removal of the corresponding sequences, no longer located in the vacuole but in the cytosol or that are, due to the addition of other signal sequences, located in other compartments.

Another possibility is the introduction of single-point mutation at positions where a modification of the amino acid sequence influences, e.g., the enzyme activity or the regulation of the enzyme. By this method mutants can be produced, for example, that possess a modified K_m -value or that are no longer subject to the regulation mechanisms that normally exist in the cell with regard to allosteric regulation or covalent modification.

Furthermore, mutants can be produced showing a modified substrate or product specificity. Also mutants can be produced showing a modified activity-temperature profile.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into

plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, NY, USA) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The term "hybridization" in the context of this invention has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from artichoke. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Seq ID No. 1 or parts of these sequences. The fragments used as hybridization probe can be synthetic fragments that were produced by means of conventional synthesis methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The molecules hybridizing to the nucleic acid molecules of the invention also comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. "Fragments" are understood to be parts of

the nucleic acid molecules that are long enough to encode one of the described proteins. The term "derivative" in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40 %, in particular an identity of at least 60 %, preferably of more than 80 % and particularly preferred of more than 90 %. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in SEQ ID No. 2 of at least 80 %, preferably of 85 % and particularly preferred of more than 90 %, 95 %, 97 % and 99 %. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore, the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes. The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as enzyme activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretic mobility, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability, pH optimum, temperature optimum.

In another preferred embodiment the invention relates to nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules. These nucleic acid molecules preferably are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can be used, for example, as primers for a PCR reaction. They can also be components of antisense constructs or of DNA molecules encoding suitable ribozymes.

The invention furthermore relates to vectors containing nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages, and other vectors usually used in the field of genetic engineering.

Preferably, the nucleic acid sequence of the invention is operatively linked to the regulatory elements in the vector of the invention that guarantee the transcription and synthesis of an RNA in prokaryotic and/or eukaryotic cells that can be translated.

The expression vectors of the invention allow the production of enzymes synthesizing short-chain fructosyl polymers in various host organisms.

The encoded enzymes can be used also outside the host organisms for the production of short-chain fructosyl polymers. Thereby, fermentative and other biotechnological methods for the production of short-chain fructosyl polymers can be used. For example, it is also imaginable to produce fructosyl polymers by means of immobilized enzymes.

According to the invention regulatory elements of the patatin B33 promoter are preferred. Other preferred promoters are the 35S CaMV promoter and the promoter of the alcohol dehydrogenase gene from *Saccharomyces cerevisiae*.

The vectors of the invention can possess further functional units effecting the stabilization of the vector in the host organism, such as a bacterial replication origin or the 2- μ DNA for the purpose of stabilization in *Saccharomyces cerevisiae*. Furthermore, "left border" and "right border" sequences of agrobacterial T-DNA can be contained, whereby a stable integration into the genome of plants is made possible.

Furthermore, the vectors of the invention can contain functional terminators, such as the terminator of the octopine synthase gene from agrobacteria.

In another embodiment the nucleic acid molecule of the invention is linked to the vector of the invention by a nucleic acid molecule encoding a functional signal.

sequence in order to transport the enzyme to various cell compartments. This modification can be, for example, the addition of an N-terminal signal sequence for secretion into the cell membrane space of higher plants but also any other modification that leads to the fusion of a signal sequence to the encoded fructosyltransferase can be the subject matter of the invention.

In a particularly preferred embodiment the invention relates to the plasmid pB33-cySST the construction of which is described in the examples (Fig. 1).

The expression of the nucleic acid molecules of the invention in prokaryotic cells, for example in *Escherichia coli*, is interesting because this way a closer characterization of the enzymatic activities of the enzymes encoding these molecules is possible.

In a further embodiment the invention relates to host cells transiently or stably containing the nucleic acid molecules or vectors of the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention.

Preferably, these cells are prokaryotic or eukaryotic cells. In particular, the invention relates to plant cells containing the vector systems of the invention or derivatives or parts thereof. Preferably, they are able to synthesize enzymes for the production of short-chain fructosyl polymers due to the fact that they have taken up the vector systems of the invention, derivatives or parts thereof. The cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to proteins being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the

culture medium. Furthermore, the invention relates to the SSTs that can be produced with the plants of the invention.

By providing the nucleic acid molecules of the invention it is now possible to produce short-chain fructosyl polymers in any organisms by means of genetic engineering, whereas up to now it had not been possible to modify plants by conventional methods, for example breeding methods, so that they are able to synthesize fructosyl polymers. By increasing the activity of the proteins of the invention, for example by overexpressing suitable nucleic acid molecules or by providing mutants that are no longer subject to the cell-specific regulation mechanisms and/or that have altered temperature dependencies with respect to their activity, it is possible to increase the yield in plants modified by genetic engineering.

Therefore, the expression of the nucleic acid molecules of the invention in plant cells in order to increase the activity of the corresponding SST or the introduction into cells normally not expressing this enzyme is now possible. Furthermore, it is possible to modify the nucleic acid molecules of the invention according to the methods known to the person skilled in the art in order to obtain SSTs of the invention that are no longer subject to the cell-specific regulation mechanisms or that have modified temperature dependencies or substrate or product specificities.

When the nucleic acid molecules are expressed in plants, the synthesized protein may be located in any compartment of the plant cell. In order to achieve the localization in a specific compartment, the sequence guaranteeing the localization in vacuole has to be deleted and, if necessary, the remaining coding region has to be linked to DNA sequences guaranteeing the localization in the specific compartment. Such sequences are known (see, e.g., Braun et al., EMBO J. 11 (1992), 3219-3227; Wolter et al., Proc. Natl. Acad. Sci. USA 85 (1988), 846-850; Sonnewald et al., Plant J. 1 (1991), 95-106). The present invention therefore also relates to transgenic plant cells that were transformed with one or several nucleotide molecule(s) of the invention as well as to transgenic plant cells originating from such transformed cells. Such cells contain one or several nucleic acid molecule(s) of the invention with it/them preferably being linked to regulatory DNA elements guaranteeing the transcription in plant cells, in particular with a promoter. Such plants can be distinguished from naturally occurring plant cells by the fact that they contain at least one nucleic acid molecule according to the

invention which does not naturally occur in these cells or by the fact that such a molecule is integrated into the genome of the cell where it does not naturally occur, i.e. in another genomic region.

The transgenic plant cells can be regenerated to whole plants using methods known to the person skilled in the art. The subject matter of the present invention relates to the plants obtainable by regeneration of the transgenic plant cells of the invention. Furthermore, the subject matter of the invention relates to plants containing the transgenic plant cells described above. The transgenic plants can basically be plants of any plant species, i.e. both monocotyledonous and dikotyledonous plants. Preferably they are crops, in particular plants that synthesize and/or store starch, such as wheat, barley, rice, maize, sugar beet, sugar cane or potato. Particularly preferred are sucrose storing plants.

The invention also relates to propagation material and harvest products of the plants of the invention, for example fruits, seeds, tubers, root stocks, seedlings, cuttings etc.

The transgenic plant cells and plants of the invention synthesize short-chain fructosyl polymers due to the expression or additional expression of at least one nucleic acid molecule of the invention.

The subject matter of the invention therefore also relates to the short-chain fructosyl polymers obtainable from the transgenic plant cells and plants of the invention as well as from the propagation material and harvest products.

The transgenic plant cells of the invention can be regenerated to whole plants according to methods known to the person skilled in the art. Therefore, the subject matter of the invention also relates to plants containing the transgenic plant cells of the invention. These plants preferably are crops, in particular plants that synthesize and/or store sucrose and/or starch. Particularly preferred is potato. The invention also relates to the propagation material of the plants of the invention, in particular tubers.

In order to express the nucleic acid molecules of the invention in sense or antisense orientation in plant cells, they are linked to regulatory DNA elements guaranteeing the transcription in plant cells. These are particularly promoters. Basically, any promoter active in plant cells is suitable for the expression.

The promoter can be selected such that the expression takes place constitutively or only in a certain tissue, at a certain stage of the plant development or at a point of time determined by external stimuli. With regard to the plant the promoter can be homologous or heterologous. Suitable promoters are, for example, the promoter of the 35S RNA of the cauliflower mosaic virus and the ubiquitin promoter from maize for a constitutive expression, particularly preferred the patatin gene promoter B33 (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) for a tuber specific expression in potato or a promoter only guaranteeing the expression in photosynthetically active tissue, for example the ST-LS1 promoter (Stockhaus et al., Proc. Natl. Acad. Sci. USA 84 (1987), 7943-7947; Stockhaus et al., EMBO J. 8 (1989), 2445-2451) or for an endosperm specific expression the HMG promoters from wheat, the USP promoter, the *Phaseolin* promoter or promoters from zein genes from maize.

Furthermore, there can be a termination sequence serving for the correct termination of the transcription as well as the addition of a poly-A tail to the transcript which is regarded as having a function for the stabilization of the transcripts. Such elements are described in the literature (cf. Gielen et al., EMBO J. 8 (1989), 23-29) and can be exchanged arbitrarily.

In order to prepare the introduction of foreign genes into higher plants there is a great number of cloning vectors available containing a replication signal for *E. coli* and a marker gene for the selection of transformed bacterial cells. Examples of such vectors are pBR322, pUC series, M13mp series, pACYC184 etc. The desired sequence can be introduced into the vector at a suitable cleavage site. The plasmid obtained is suitable for the transformation of *E. coli* cells. Transformed *E. coli* cells are cultivated in a suitable medium, then harvested and lysed. The plasmid is regenerated. Usually, restriction analyses, gel electrophoreses and other biochemical or molecular biological methods are used as analysis methods for the characterization of the regenerated plasmid DNA. After every manipulation the plasmid DNA can be cleaved and the regenerated DNA fragments linked to other DNA sequences. Every plasmid DNA sequence can be cloned into the same or other plasmids.

For the introduction of DNA into a plant host cell a great number of methods are available. These methods comprise the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as means for transformation;

the fusion of protoplasts, the injection, the electroporation of DNA, the introduction of DNA by means of the biolistic methods as well as further possibilities.

For the injection and electroporation of DNA in plant cells there are no specific requirements for the plasmids used. Simple plasmids such as pUC derivatives can be used. If whole plants are to be regenerated from such transformed cells, there should be a selectable marker.

Depending on the method for the introduction of desired genes into the plant cell further DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, at least the right border, often, however, the right and left border of the Ti and Ri plasmid T-DNA have to be linked as flanking region to the genes to be introduced.

If agrobacteria are used for the transformation, the DNA to be introduced has to be cloned into specific plasmids, either into an intermediary vector or into a binary vector. The intermediary vectors can be integrated into the Ti or Ri plasmid of the agrobacteria due to sequences that are homologous to sequences in the T-DNA by homologous recombination. The Ti or Ri plasmid furthermore contains the *vir* region necessary for the transfer of the T-DNA. Intermediary vectors cannot replicate in agrobacteria. By means of a helper plasmid the intermediary vector can be transferred to *Agrobacterium tumefaciens* (conjugation). Binary vectors can replicate both in *E. coli* and in agrobacteria. They contain a selection marker gene and a linker or polylinker framed by the right and left T-DNA border region. They can be transformed directly into the agrobacteria (Holsters et al., Mol. Gen. Genet. 163 (1978), 181-187). The agrobacterium serving as a host cell should contain a plasmid carrying a *vir* region. The *vir* region is necessary for the transfer of the T-DNA into the plant cell. There may be additional T-DNA. The agrobacterium transformed such is used for the transformation of plant cells. The use of T-DNA for the transformation of plant cells has extensively been examined and described in EP-A-120 516; Hoekema: The Binary Plant Vector System, Offsetdrukkerij Kanters B.V., Alblasterdam (1985); Chapter V, Fraley et al., Crit. Rev. Plant. Sci., 4, 1-46 and An et al., EMBO J. 4 (1985), 277-287. For the transfer of the DNA into the plant cell plant explants can be co-cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. From the infected plant material (e.g., pieces of leaf, stem segments, roots, but also protoplasts or plant cells cultivated by suspension) whole plants can be regenerated in a suitable medium.

which may contain antibiotics or biozides for the selection of transformed cells. The plants obtained this way can be examined for the presence of the introduced DNA. Other possibilities of introducing foreign DNA using the biolistic methods or by protoplast transformation are known (cf., e.g., Willmitzer, L., 1993 Transgenic plants. In: Biotechnology, A Multi-Volume Comprehensive Treatise (H.J. Rehm, G. Reed, A. Pühler, P. Stadler, eds.), Vol. 2, 627-659, VCH Weinheim-New York-Basel-Cambridge).

Alternative systems for the transformation of monocotyledonous plants are the transformation by means of the biolistic approach, the electrically or chemically induced introduction of DNA into protoplasts, the electroporation of partially permeabilized cells, the macroinjection of DNA into flowers, the microinjection of DNA into microspores and pro-embryos, the introduction of DNA into germinating pollen and the introduction of DNA into embryos by swelling (for review: Potrykus, *Physiol. Plant* (1990), 269-273).

While the transformation of dicotyledonous plants via Ti plasmid vector systems with the help of *Agrobacterium tumefaciens* is well-established, more recent research work indicates that also monocotyledonous plants are accessible for transformation by means of vectors based on *Agrobacterium* (Chan et al., *Plant Mol. Biol.* 22 (1993), 491-506; Hiei et al., *Plant J.* 6 (1994), 271-282; Bytebier et al., *Proc. Natl. Acad. Sci. USA* 84 (1987), 5345-5349; Raineri et al., *Bio/Technology* 8 (1990), 33-38; Gould et al., *Plant Physiol.* 95 (1991), 426-434; Mooney et al., *Plant, Cell Tiss. & Org. Cult.* 25 (1991), 209-218; Li et al., *Plant Mol. Biol.* 20 (1992), 1037-1048).

Three of the above-mentioned transformation systems could be established for various cereals: the electroporation of tissues, the transformation of protoplasts and the DNA transfer by particle bombardment in regenerative tissue and cells (for review: Jähne et al., *Euphytica* 85 (1995), 35-44).

The transformation of wheat has been frequently described in the literature (for review: Maheshwari et al., *Critical Reviews in Plant Science* 14 (2) (1995), 149-178).

The invention also relates to plants containing at least one, preferably a number of cells containing the vector systems of the invention or derivatives or parts thereof and being able to synthesize enzymes for the production of short-chain fructosyl polymers due to the introduction of the vector systems, derivatives or parts of the vector systems

of the invention. The invention also provides plants of many species, genres, families, orders and classes that are able to synthesize enzymes for the production of short-chain fructosyl polymers due to the introduced vector systems or derivatives or parts thereof. Since the known plants are not able to only produce short-chain fructosyl polymers, it is easy to check whether the method has been successfully performed, for example by chromatographic analysis of the sugars containing fructose. They are advantageous vis-à-vis the few plants containing fructosyl polymers since there is a defined molecular size, i.e. the size of the short-chain fructosyl polymer. Furthermore, a localization in the various cell compartments and various organs as well as an increase of the expression ratio and therefore of the yield is possible.

In another embodiment the invention relates to methods for the production of short-chain fructosyl polymers comprising:

- (a) contacting sucrose or an equivalent substrate with an SST of the invention under conditions allowing the conversion to short-chain fructosyl polymers; and
- (b) obtaining the fructosyl polymers produced this way.

The nature of the produced fructosyl polymers depends on the enzymatic specificity of the fructosyl transferase. When an SST of the invention is used, preferably kestose but also nystose and fructosylnystose are produced.

Furthermore, the invention relates to the fructosyl polymers produced from a plant cell or plant of the invention or from the propagation material or harvest product of plants or plant cells of the invention or obtained according to the above-described method of the invention. These fructosyl polymers can preferably be used for the production of food such as baked goods or pasta. Preferably, these fructosyl polymers can be used for increasing the viscosity in aqueous systems, as detergents, as suspending agents or for accelerating the sedimentation process and complexing but also for binding water.

The figures show:

Figure 1 shows the construction of the plasmid pB33-cySST.

Vector:	pBinB33 (derivative of pBin19; Bevan, 1984, Nucl Acids Res 12: 8711)
promoter:	B33 promoter (Rocha-Sosa et al., 1989, EMBO J 8: 23-29)
donor:	<i>Solanum tuberosum</i>
coding region:	SST gene from <i>Cynara scolymus</i>
orientation:	sense
terminator:	Polyadenylation signal of the octopin synthase gene from <i>A. tumefaciens</i> plasmid pTiACH5 (Gielen et al., 1984, EMBO J 3: 835-846)
donator:	<i>Agrobacterium tumefaciens</i>
resistance:	kanamycin

Figure 2 shows the analysis of the soluble sugars in the tubers of transgenic plants that were produced using the vector system pB33-cySST. The short-chain fructosyl polymers (in particular 1-kestose) produced due to the genetic modification have been labeled.

Figure 3 shows the analysis of the soluble sugars in transgenic plants that were produced using the vector system pB33-cySST and p35S-cySST, respectively, compared to wildtype plants.

Example 1: Identification, isolation and characterization of a cDNA encoding a sucrose dependent sucrose-fructosyltransferase from artichoke (*Cynara scolymus*)

Total RNA was isolated from blossom discs of artichoke (Sambrook et al., see supra). Poly(A)⁺ mRNA was isolated using the mRNA isolation system PolyATtract (Promega Corporation, Madison, WI, USA). Complementary DNA (cDNA) was produced from 5

µg of this RNA by means of the ZAP-cDNA synthesis kit of Stratagene according to the manufacturer's instructions. 2×10^6 independent recombinant phages were obtained. The amplified cDNA library was screened by conventional methods with a DNA fragment labeled with ^{32}P and corresponding to the 3'-terminal of the 6-SFT cDNA (Sprenger et al., Proc. Natl. Acad. Sci. USA 92 (1995), 11652) having a length of 392 bp. This fragment was obtained from the complete RNA by RT-PCR (RT-PCR Kit, Stratagene, Heidelberg, Germany) as matrix from light-induced (72 hours) primary leaves from barley. Positive clones were further examined.

Example 2: Sequence analysis of the cDNA insertion of the plasmid pCy21

The plasmid DNA was isolated from the clone pCy21. The sequence of the cDNA insertion was determined by conventional methods by means of the dideoxynucleotide method (Sanger et al., Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467).

The insertion of the clone pCy21 is a DNA of 2055 bp. The nucleotide sequence is depicted in Seq ID No. 1. The corresponding amino acid sequence is depicted in Seq ID No. 2.

A sequence analysis and a comparison with already published sequences showed that the sequence depicted in Seq ID No. 1 is novel and comprises a coding region showing homologies to SSTs from other organisms.

Example 3: Production of the plasmid pB33-cySST and introduction of the plasmid into the genome of potato

The plasmid pB33-cySST contains three fragments A, B and C in the binary vector pBin19 (Bevan, 1984, Nucl Acids Res 12: 8711, modified according to Becker, 1990, Nucl Acids Res 18: 203) (cf. Fig. 1). Fragment A contains the B33 promoter of the patatin gene b33 of potato. It contains a DraI fragment (position -1512 to position +14) of the patatin gene B33 (Rocha-Sosa et al., 1989, EMBO J 8:23-29), which is inserted between the EcoRI and the SacI cleavage site of the polylinker of pBin19-Hyg. Fragment B contains the coding region of the sequence depicted in SEQ ID No. 1. Fragment B was obtained as NotI fragment with blunt ends from the vector pBluescript

SK, in which it is inserted into the EcoRI cleavage site via an EcoRI/Not I linker sequence. Fragment C contains the polyadenylation signal of the gene 3 of the T-DNA of the Ti plasmid pTi ACH 5 (Gielen et al (1984); EMBO J. 3, 835-846) nucleotides 11749 - 11939, which was isolated as Pvu II-Hind III fragment from the plasmid pAGV 40 (Herrera-Estrella et al (1983) Nature 303, 209 - 213) and cloned between the SphI and the Hind III cleavage site of the polylinker of pBin19-Hyg after the addition of Sph I linkers to the Pvu II cleavage site. The plasmid pB33-cySST has a size of approx. 14 kb. The plasmid was introduced into agrobacteria (Höfgen and Willmitzer, Nucleic Acids Res. 16 (1988), 9877).

The plasmid pB33-cySST was introduced into potato plants via the gene transfer induced by *Agrobacterium* according to the above-described conventional methods. Intact plants were regenerated from transformed cells. From regenerated plants enzyme extracts were obtained and examined for the presence of fructosyl polymers. The analysis was carried out as described in Röber (Planta 199, 528-536). The analysis of the tubers of a number of transformed plants transformed with this vector clearly showed the presence of short-chain fructosyl polymers, in particular 1-kestose, which can be put down to the expression of the SST gene of the invention (cf. Fig. 2).

Example 4 Analysis of soluble sugar in wildtype and SST containing transgenic plants

Transgenic plants containing vectors pB33-cySST and 35S-cySST (having the coding region of SEQ ID No. 1 under the control of the 35S promoter) were generated as described in Example 3. Extracts were obtained from transgenic plants and wildtype plants and examined for the presence of fructosyl polymers, see Example 3. HPAEC-analysis shown in Figure 3 demonstrates the production of oligofructanes. The results are summarized in Table 1, below.

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Table 1

Soluble sugars (sucrose and oligofructane) in wildtype and transgenic plants

line	sucrose	1-kestose	nystose	F-nystose
WT 1 (Désirée)	2,09	-	-	-
WT 2 (Désirée)	1,67	-	-	-
B33-cySST 6	2,26	3,58	1,60	-
B33-cySST 54	5,13	3,06	2,90	0,23
35S-cySST 18	4,08	4,05	1,51	0,12
35S-cySST 22	4,80	4,14	2,19	< 0,1

Values in g carbohydrate per kg fresh weight

As is evident from Figure 3 and Table 1, supra, the content of fructosyl polymers, in particular 1-kestose exceeds the content of sucrose. Thus, the experiments performed in accordance with the present invention demonstrate the usefulness of the nucleic acid molecules of the invention for the production of fructosyl polymers in transgenic plants.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Max-Planck-Gesellschaft zur Foerderung der
Wissenschaften e.V.
(B) STREET: none
(C) CITY: Berlin
(E) COUNTRY: DE
(F) ZIP CODE: NONE

(ii) TITLE OF THE INVENTION: Nucleic acid molecules encoding enzymes
having fructosyl polymerase activity

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2226 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) IMMEDIATE SOURCE:

- (A) ORGANISM: Cynara Scolymus

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 8..1918

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CCACCAC	ATG	GCT	TCC	TCT	ACC	ACC	ACC	CCA	CTC	CTC	CCT	CAC	CAC	CAC	49	
Met	Ala	Ser	Ser	Thr	Thr	Thr	Pro	Leu	Leu	Pro	His	His	His			
1				5					10							
CTT	CAG	AAC	CCG	CAA	CAA	CTC	GCC	GGA	TCT	CCG	GCA	GCT	CAT	CGT	CTA	97
Leu	Gln	Asn	Pro	Gln	Gln	Leu	Ala	Gly	Ser	Pro	Ala	Ala	His	Arg	Leu	
15				20				25						30		
TCC	CGA	CCC	ACA	CTC	CTT	TCT	GGG	ATC	CTT	GTT	TCG	GTC	CTA	GTC	ATC	145
Ser	Arg	Pro	Thr	Leu	Ser	Gly	Ile	Leu	Val	Ser	Val	Leu	Val	Ile		
				35			40					45				

TGT GCT CTC GTT GCT GTA ATC CAC AAC CAA TCA CAG CAA CCC TAC CAT	193
Cys Ala Leu Val Ala Val Ile His Asn Gln Ser Gln Gln Pro Tyr His	
50 55 60	
GAC GGC GGA GCT AAA CCC TCC TCC TCC GCC GCT ACC ACC ACC TTC CCA	241
Asp Gly Gly Ala Lys Pro Ser Ser Ser Ala Ala Thr Thr Thr Phe Pro	
65 70 75	
ACA GCG TCG CCA GAA GCT GGT TTG AAA CGG TTT CCC ATT GAG TTG AAA	289
Thr Ala Ser Pro Glu Ala Gly Leu Lys Arg Phe Pro Ile Glu Leu Lys	
80 85 90	
ACG AAT GCT GAG GTT GAG TGG CAA CGC TCG GCT TAC CAT TTT CAG CCC	337
Thr Asn Ala Glu Val Glu Trp Gln Arg Ser Ala Tyr His Phe Gln Pro	
95 100 105 110	
GAT AAG AAC TAC ATT AGC GAT CCT GAT GGC CCA ATG TAT CAC ATG GGG	385
Asp Lys Asn Tyr Ile Ser Asp Pro Asp Gly Pro Met Tyr His Met Gly	
115 120 125	
TGG TAT CAT CTC TTC TAT CAG TAC AAT CCA GAG TCT GCC ATC TGG GGG	433
Trp Tyr His Leu Phe Tyr Gln Tyr Asn Pro Glu Ser Ala Ile Trp Gly	
130 135 140	
AAC ATC ACA TGG GGC CAC TCC GTA TCC AAA GAC ATG ATC AAC TGG TTC	481
Asn Ile Thr Trp Gly His Ser Val Ser Lys Asp Met Ile Asn Trp Phe	
145 150 155	
CAT CTC CCC TTC GCC ATG GTC CCT GAC CAA TGG TAC GAT ATC GAA GGT	529
His Leu Pro Phe Ala Met Val Pro Asp Gln Trp Tyr Asp Ile Glu Gly	
160 165 170	
GTC ATG ACC GGC TCC GCC ACC GTC CTC CCT GAC GGT CAG ATC ATC ATG	577
Val Met Thr Gly Ser Ala Thr Val Leu Pro Asp Gly Gln Ile Ile Met	
175 180 185 190	
CTC TAC ACC GGC AAC GCG TAC GAT CTC TCG CAA CTG CAA TGC TTA GCA	625
Leu Tyr Thr Gly Asn Ala Tyr Asp Leu Ser Gln Leu Gln Cys Leu Ala	
195 200 205	
TAT GCC GTC AAC TCG TCT GAT CCC CTC CTC CTC GAT TGG AAA AAG TAC	673
Tyr Ala Val Asn Ser Ser Asp Pro Leu Leu Leu Asp Trp Lys Lys Tyr	
210 215 220	
GAG GGA AAT CCC ATC TTG TTC CCA CCT CCT GGG GTG GGA TAC AAG GAT	721
Glu Gly Asn Pro Ile Leu Phe Pro Pro Pro Gly Val Gly Tyr Lys Asp	
225 230 235	
TTT CGG GAC CCA TCT ACA CTG TGG TTG GGT CCC GAT GGT GAA TAC AGA	769
Phe Arg Asp Pro Ser Thr Leu Trp Leu Gly Pro Asp Gly Glu Tyr Arg	
240 245 250	
ATG GTA ATG GGG TCC AAG CAT AAC GAG ACC ATC GGT TGT GCC TTG ATT	817
Met Val Met Gly Ser Lys His Asn Glu Thr Ile Gly Cys Ala Leu Ile	
255 260 265 270	
TAC CAT ACC ACT AAT TTT ACG CAT TTC GAG CTC AAG GAA GAG GTG CTT	865
Tyr His Thr Thr Asn Phe Thr His Phe Glu Leu Lys Glu Glu Val Leu	
275 280 285	

CAC GCC GTT CCC CAC ACG GGT ATG TGG GAA TGT GTG GAT CTT TAT CCG His Ala Val Pro His Thr Gly Met Trp Glu Cys Val Asp Leu Tyr Pro 290 295 300	913
GTA TCC ACC ACG CAC ACA AAC GGG TTG GAC ATG GTG GAT AAC GGG CCG Val Ser Thr Thr His Thr Asn Gly Leu Asp Met Val Asp Asn Gly Pro 305 310 315	961
AAT GTG AAG CAT GTG TTG AAA CAA AGT GGG GAT GAA GAT CGA CAT GAT Asn Val Lys His Val Leu Lys Gln Ser Gly Asp Glu Asp Arg His Asp 320 325 330	1009
TGG TAT GCG CTC GGG ACT TAT GAC GTC GTG AAT GAT AAG TGG TAT CCA Trp Tyr Ala Leu Gly Thr Tyr Asp Val Val Asn Asp Lys Trp Tyr Pro 335 340 345 350	1057
GAT GAC CCT GAA AAC GAT GTG GGT ATC GGG TTA AGA TAC GAT TTC GGA Asp Asp Pro Glu Asn Asp Val Gly Ile Gly Leu Arg Tyr Asp Phe Gly 355 360 365	1105
AAG TTT TAT GCG TCA AAG ACG TTC TAC GAC CAA CAT AAG AAG AGA CGG Lys Phe Tyr Ala Ser Lys Thr Phe Tyr Asp Gln His Lys Lys Arg Arg 370 375 380	1153
GTC CTT TGG GGT TAC GTT GGA GAA ACC GAT CCC CCT AAA TAC GAC GTT Val Leu Trp Gly Tyr Val Gly Glu Thr Asp Pro Pro Lys Tyr Asp Val 385 390 395	1201
TAC AAG GGA TGG GCT AAC ATT TTG AAC ATT CCA AGG ACC ATA GTT TTG Tyr Lys Gly Trp Ala Asn Ile Leu Asn Ile Pro Arg Thr Ile Val Leu 400 405 410	1249
GAC ACG AAA ACG AAT ACC AAT TTG ATT CAA TGG CCA ATT GCG GAA GTC Asp Thr Lys Thr Asn Thr Asn Leu Ile Gln Trp Pro Ile Ala Glu Val 415 420 425 430	1297
GAA AAC TTG AGA TCG AAT AAA TAC AAT GAA TTC AAA GAC GTG GAG CTG Glu Asn Leu Arg Ser Asn Lys Tyr Asn Glu Phe Lys Asp Val Glu Leu 435 440 445	1345
AAA CCG GGA TCA CTG ATT CCG CTC GAG ATA GGC ACA GCA ACA CAG TTG Lys Pro Gly Ser Leu Ile Pro Leu Glu Ile Gly Thr Ala Thr Gln Leu 450 455 460	1393
GAT ATA ACT GCG ACA TTC GAA GTT GAT CAA ACG ATG TTG GAA TCG ACG Asp Ile Thr Ala Thr Phe Glu Val Asp Gln Thr Met Leu Glu Ser Thr 465 470 475	1441
CTT GAA GCC GAT GTT TTG TTC AAT TGT ACG ACC AGT GAA GGT TCA GCC Leu Glu Ala Asp Val Leu Phe Asn Cys Thr Thr Ser Glu Gly Ser Ala 480 485 490	1489
GGG AGA GGG GTG TTG GGG CCA TTT GGA CTG GTG GTT CTA GCT GAT GCC Gly Arg Gly Val Leu Gly Pro Phe Gly Leu Val Val Leu Ala Asp Ala 495 500 505 510	1537

GAA CGA TCT GAG CAA CTT CCT GTG TAT TTC TAT ATA GCA AAA GAC ACC Glu Arg Ser Glu Gln Leu Pro Val Tyr Phe Tyr Ile Ala Lys Asp Thr 515 520 525	1585
GAT GGA TCC TCA AAA ACT TAC TTC TGT GCC GAT GAA TCA AGA TCA TCG Asp Gly Ser Ser Lys Thr Tyr Phe Cys Ala Asp Glu Ser Arg Ser Ser 530 535 540	1633
AAC GAT GTA GAC ATA GGG AAA TGG GTG TAC GGA AGC AGT GTT CCT GTT Asn Asp Val Asp Ile Gly Lys Trp Val Tyr Gly Ser Ser Val Pro Val 545 550 555	1681
CTA GAA GGC GAA AAA TTC AAC ATG AGG TTG CTG GTG GAT CAT TCA ATT Leu Glu Gly Glu Lys Phe Asn Met Arg Leu Leu Val Asp His Ser Ile 560 565 570	1729
GTC GAA GGC TTC GCA CAA GGA GGC AGA ACG GTG GTG ACA TCA AGA GTG Val Glu Gly Phe Ala Gln Gly Gly Arg Thr Val Val Thr Ser Arg Val 575 580 585 590	1777
TAT CCG GCG AAG GCG ATC TAC GGC GCT GCA AAG TTA TTT TTG TTC AAC Tyr Pro Ala Lys Ala Ile Tyr Gly Ala Ala Lys Leu Phe Leu Phe Asn 595 600 605	1825
AAC GCC ACC GGA ATC AGC GTG AAG GCA TCT CTC AAG ATC TGG AAA ATG Asn Ala Thr Gly Ile Ser Val Lys Ala Ser Leu Lys Ile Trp Lys Met 610 615 620	1873
AAG GAA GCA CAA CTG GAT CCA TTC CCT CTT TCT GGA TGG AGT TCT Lys Glu Ala Gln Leu Asp Pro Phe Pro Leu Ser Gly Trp Ser Ser 625 630 635	1918
TGATGATGAT GATGATTAAG AACTCATTTT ATGAAGATGA TGATTAAGAA CTCATTTTCAT	1978
GATGATGATG ATGATTCCAG TTTATATGCG TACCCTGTTC CCTTTACCTG TATGTGGTGG	2038
TGGTGGTGAA ATATGGTTAG CATGATTCCG GGTGGCGAG GGCAATATGG TAATTTACTA	2098
TCGCTGTAGT AGTACTCCAC TTGTGAGATT ATATTTTCATA AATTCAATTA TTATTCCTGT	2158
TTACAACCTT TTTTATTGTA TCATACCACC CATTGAATCC CATCATGTTC AATTAGTGT	2218
GCAAAAAA	2226

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:: 637 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Ser Ser Thr Thr Thr Pro Leu Leu Pro His His His Leu Gln
1 5 10 15

Asn Pro Gln Gln Leu Ala Gly Ser Pro Ala Ala His Arg Leu Ser Arg
 20 25 30
 Pro Thr Leu Leu Ser Gly Ile Leu Val Ser Val Leu Val Ile Cys Ala
 35 40 45
 Leu Val Ala Val Ile His Asn Gln Ser Gln Gln Pro Tyr His Asp Gly
 50 55 60
 Gly Ala Lys Pro Ser Ser Ser Ala Ala Thr Thr Thr Phe Pro Thr Ala
 65 70 75 80
 Ser Pro Glu Ala Gly Leu Lys Arg Phe Pro Ile Glu Leu Lys Thr Asn
 85 90 95
 Ala Glu Val Glu Trp Gln Arg Ser Ala Tyr His Phe Gln Pro Asp Lys
 100 105 110
 Asn Tyr Ile Ser Asp Pro Asp Gly Pro Met Tyr His Met Gly Trp Tyr
 115 120 125
 His Leu Phe Tyr Gln Tyr Asn Pro Glu Ser Ala Ile Trp Gly Asn Ile
 130 135 140
 Thr Trp Gly His Ser Val Ser Lys Asp Met Ile Asn Trp Phe His Leu
 145 150 155 160
 Pro Phe Ala Met Val Pro Asp Gln Trp Tyr Asp Ile Glu Gly Val Met
 165 170 175
 Thr Gly Ser Ala Thr Val Leu Pro Asp Gly Gln Ile Ile Met Leu Tyr
 180 185 190
 Thr Gly Asn Ala Tyr Asp Leu Ser Gln Leu Gln Cys Leu Ala Tyr Ala
 195 200 205
 Val Asn Ser Ser Asp Pro Leu Leu Leu Asp Trp Lys Lys Tyr Glu Gly
 210 215 220
 Asn Pro Ile Leu Phe Pro Pro Pro Gly Val Gly Tyr Lys Asp Phe Arg
 225 230 235 240
 Asp Pro Ser Thr Leu Trp Leu Gly Pro Asp Gly Glu Tyr Arg Met Val
 245 250 255
 Met Gly Ser Lys His Asn Glu Thr Ile Gly Cys Ala Leu Ile Tyr His
 260 265 270
 Thr Thr Asn Phe Thr His Phe Glu Leu Lys Glu Glu Val Leu His Ala
 275 280 285
 Val Pro His Thr Gly Met Trp Glu Cys Val Asp Leu Tyr Pro Val Ser
 290 295 300
 Thr Thr His Thr Asn Gly Leu Asp Met Val Asp Asn Gly Pro Asn Val
 305 310 315 320
 Lys His Val Leu Lys Gln Ser Gly Asp Glu Asp Arg His Asp Trp Tyr
 325 330 335

Ala Leu Gly Thr Tyr Asp Val Val Asn Asp Lys Trp Tyr Pro Asp Asp
 340 345 350

Pro Glu Asn Asp Val Gly Ile Gly Leu Arg Tyr Asp Phe Gly Lys Phe
 355 360 365

Tyr Ala Ser Lys Thr Phe Tyr Asp Gln His Lys Lys Arg Arg Val Leu
 370 375 380

Trp Gly Tyr Val Gly Glu Thr Asp Pro Pro Lys Tyr Asp Val Tyr Lys
 385 390 395 400

Gly Trp Ala Asn Ile Leu Asn Ile Pro Arg Thr Ile Val Leu Asp Thr
 405 410 415

Lys Thr Asn Thr Asn Leu Ile Gln Trp Pro Ile Ala Glu Val Glu Asn
 420 425 430

Leu Arg Ser Asn Lys Tyr Asn Glu Phe Lys Asp Val Glu Leu Lys Pro
 435 440 445

Gly Ser Leu Ile Pro Leu Glu Ile Gly Thr Ala Thr Gln Leu Asp Ile
 450 455 460

Thr Ala Thr Phe Glu Val Asp Gln Thr Met Leu Glu Ser Thr Leu Glu
 465 470 475 480

Ala Asp Val Leu Phe Asn Cys Thr Thr Ser Glu Gly Ser Ala Gly Arg
 485 490 495

Gly Val Leu Gly Pro Phe Gly Leu Val Val Leu Ala Asp Ala Glu Arg
 500 505 510

Ser Glu Gln Leu Pro Val Tyr Phe Tyr Ile Ala Lys Asp Thr Asp Gly
 515 520 525

Ser Ser Lys Thr Tyr Phe Cys Ala Asp Glu Ser Arg Ser Ser Asn Asp
 530 535 540

Val Asp Ile Gly Lys Trp Val Tyr Gly Ser Ser Val Pro Val Leu Glu
 545 550 555 560

Gly Glu Lys Phe Asn Met Arg Leu Leu Val Asp His Ser Ile Val Glu
 565 570 575

Gly Phe Ala Gln Gly Gly Arg Thr Val Val Thr Ser Arg Val Tyr Pro
 580 585 590

Ala Lys Ala Ile Tyr Gly Ala Ala Lys Leu Phe Leu Phe Asn Asn Ala
 595 600 605

Thr Gly Ile Ser Val Lys Ala Ser Leu Lys Ile Trp Lys Met Lys Glu
 610 615 620

Ala Gln Leu Asp Pro Phe Pro Leu Ser Gly Trp Ser Ser
 625 630 635

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1911 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1911

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATG GCA AGC TCT ACG ACT ACA CCG TTG TTA CCG CAC CAC CAT TTG CAG	48
Met Ala Ser Ser Thr Thr Thr Pro Leu Leu Pro His His His Leu Gln	
640 645 650	
AAT CCT CAG CAG TTG GCT GGA AGT CCA GCT GCA CAC AGG TTG AGT CGT	96
Asn Pro Gln Gln Leu Ala Gly Ser Pro Ala Ala His Arg Leu Ser Arg	
655 660 665	
CCT ACT CTT TTG AGT GGT ATA TTG GTA AGT GTA CTG GTC ATC TGC GCA	144
Pro Thr Leu Leu Ser Gly Ile Leu Val Ser Val Leu Val Ile Cys Ala	
670 675 680 685	
TTG GTC GCA GTT ATA CAT AAT CAG TCT CAA CAG CCA TAC CAT GAT GGT	192
Leu Val Ala Val Ile His Asn Gln Ser Gln Gln Pro Tyr His Asp Gly	
690 695 700	
GGT GCC AAG CCT AGC TCT AGC GCT GCC ACG ACT ACT TTT CCT ACA GCC	240
Gly Ala Lys Pro Ser Ser Ser Ala Ala Thr Thr Thr Phe Pro Thr Ala	
705 710 715	
AGC CCT GAA GCA GGA TTG AAA AGA TTC CCT ATC GAA CTC AAG ACC AAC	288
Ser Pro Glu Ala Gly Leu Lys Arg Phe Pro Ile Glu Leu Lys Thr Asn	
720 725 730	
GCA GAA GTC GAG TGG CAG AGA AGT GCA TAC CAC TTC CAG CCA GAT AAG	336
Ala Glu Val Glu Trp Gln Arg Ser Ala Tyr His Phe Gln Pro Asp Lys	48
735 740 745	
AAC TAT ATC TCA GAC CCA GAC GGG CCT ATG TAC CAT ATG GGT TGG TAC	384
Asn Tyr Ile Ser Asp Pro Asp Gly Pro Met Tyr His Met Gly Trp Tyr	
750 755 760 765	
CAC TTA TTC TAC CAA TAT AAT CCA GAG AGT GCA ATA TGG GGA AAT ATA	432
His Leu Phe Tyr Gln Tyr Asn Pro Glu Ser Ala Ile Trp Gly Asn Ile	
770 775 780	
ACT TGG GGT CAT AGC GTT AGC AAG GAT ATG ATT AAT TGG TTT CAC TTG	480
Thr Trp Gly His Ser Val Ser Lys Asp Met Ile Asn Trp Phe His Leu	192
785 790 795	
CCA TTT GCG ATG GTC CCA GAT CAA TGG TAT GAT ATT GAG GGC GTT ATG	528

Pro	Phe	Ala	Met	Val	Pro	Asp	Gln	Trp	Tyr	Asp	Ile	Glu	Gly	Val	Met	
	800						805					810				
ACT	GGA	AGC	GCA	ACT	GTT	TTG	CCA	GAC	GGA	CAG	ATC	ATT	ATG	TTG	TAT	576
Thr	Gly	Ser	Ala	Thr	Val	Leu	Pro	Asp	Gly	Gln	Ile	Ile	Met	Leu	Tyr	
	815					820					825					
ACC	GGT	AAT	GCA	TAC	GAC	TTG	AGT	CAG	TTG	CAG	TGT	CTC	GCC	TAT	GCC	624
Thr	Gly	Asn	Ala	Tyr	Asp	Leu	Ser	Gln	Leu	Gln	Cys	Leu	Ala	Tyr	Ala	
	830				835					840					845	
GTT	AAT	AGC	AGC	GAC	CCC	TTG	TTG	CTC	GAT	TGG	AAG	AAG	TAC	GAG	GGC	672
Val	Asn	Ser	Ser	Asp	Pro	Leu	Leu	Leu	Asp	Trp	Lys	Lys	Tyr	Glu	Gly	
				850					855					860		
AAT	CCG	ATT	CTC	TTT	CCG	CCT	CCT	GGC	GTC	GGA	TAT	AAA	GAT	TTC	AGA	720
Asn	Pro	Ile	Leu	Phe	Pro	Pro	Pro	Gly	Val	Gly	Tyr	Lys	Asp	Phe	Arg	
			865					870					875			
GAT	CCC	AGT	ACT	CTC	TGG	CTC	GGT	CCA	GAC	GGA	GAG	TAC	CGT	ATG	GTC	768
Asp	Pro	Ser	Thr	Leu	Trp	Leu	Gly	Pro	Asp	Gly	Glu	Tyr	Arg	Met	Val	
		880					885					890				
ATG	GGC	AGC	AAA	CAC	AAT	GAA	ACA	ATC	GGG	TGC	GCA	CTC	ATC	TAT	CAC	816
Met	Gly	Ser	Lys	His	Asn	Glu	Thr	Ile	Gly	Cys	Ala	Leu	Ile	Tyr	His	
	895					900					905					
ACG	ACA	AAC	TTC	ACG	CAC	TTC	GAG	CTC	AAG	GAA	GAA	GTC	TTA	CAC	GCT	864
Thr	Thr	Asn	Phe	Thr	His	Phe	Glu	Leu	Lys	Glu	Glu	Val	Leu	His	Ala	
	910				915					920					925	
GTT	CCT	CAC	ACA	GGA	ATG	TGG	GAG	TGC	GTC	GAC	TTA	TAT	CCC	GTC	AGT	912
Val	Pro	His	Thr	Gly	Met	Trp	Glu	Cys	Val	Asp	Leu	Tyr	Pro	Val	Ser	
				930					935					940		
ACT	ACT	CAT	ACG	AAT	GGC	TTG	GAT	ATG	GTC	GAC	AAT	GGT	CCC	AAC	GTC	960
Thr	Thr	His	Thr	Asn	Gly	Leu	Asp	Met	Val	Asp	Asn	Gly	Pro	Asn	Val	
			945					950					955			
AAA	CAT	GTC	CTC	AAG	CAG	TCC	GGC	GAC	GAG	GAC	AGG	CAC	GAC	TGG	TAC	1008
Lys	His	Val	Leu	Lys	Gln	Ser	Gly	Asp	Glu	Asp	Arg	His	Asp	Trp	Tyr	
		960					965					970				
GCT	TTA	GGT	ACA	TAT	GAC	GTC	GTC	AAC	GAC	AAA	TGG	TAT	CCC	GAC	GAT	1056
Ala	Leu	Gly	Thr	Tyr	Asp	Val	Val	Asn	Asp	Lys	Trp	Tyr	Pro	Asp	Asp	
	975					980				985						
CCC	GAG	AAC	GAC	GTC	GGA	ATT	GGC	CTT	CGT	TAC	GAC	TTC	GGC	AAG	TTC	1104
Pro	Glu	Asn	Asp	Val	Gly	Ile	Gly	Leu	Arg	Tyr	Asp	Phe	Gly	Lys	Phe	
	990				995					1000					1005	
TAC	GCC	AGT	AAA	ACA	TTC	TAC	GAT	CAG	CAC	AAA	AAA	CGT	CGT	GTT	TTA	1152
Tyr	Ala	Ser	Lys	Thr	Phe	Tyr	Asp	Gln	His	Lys	Lys	Arg	Arg	Val	Leu	
				1010					1015					1020		
TGG	GGA	TAC	GTC	GGC	GAG	ACG	GAC	CCG	CCC	AAA	TAC	GAT	GTC	TAC	AAA	1200
Trp	Gly	Tyr	Val	Gly	Glu	Thr	Asp	Pro	Pro	Lys	Tyr	Asp	Val	Tyr	Lys	
			1025					1030					1035			
GGT	TGG	GCA	AAT	ATC	CTC	AAC	ATA	CCT	CGC	ACT	ATT	GTC	CTC	GAT	ACG	1248

Gly	Trp	Ala	Asn	Ile	Leu	Asn	Ile	Pro	Arg	Thr	Ile	Val	Leu	Asp	Thr	
1040						1045						1050				
AAG	ACA	AAC	ACG	AAC	CTC	ATA	CAG	TGG	CCT	ATT	GCC	GAG	GTG	GAG	AAT	1296
Lys	Thr	Asn	Thr	Asn	Leu	Ile	Gln	Trp	Pro	Ile	Ala	Glu	Val	Glu	Asn	
1055						1060						1065				
TTA	CGT	AGC	AAC	AAA	TAC	AAC	GAG	TTC	AAG	GAT	GTG	GAA	TTG	AAG	CCT	1344
Leu	Arg	Ser	Asn	Lys	Tyr	Asn	Glu	Phe	Lys	Asp	Val	Glu	Leu	Lys	Pro	
1070						1075						1080				1085
GGA	AGT	TTG	ATT	CCG	TTA	GAA	ATC	GGT	ACT	GCT	ACT	CAA	CTC	GAC	ATC	1392
Gly	Ser	Leu	Ile	Pro	Leu	Glu	Ile	Gly	Thr	Ala	Thr	Gln	Leu	Asp	Ile	
1090						1095						1100				
ACC	GCT	ACT	TTT	GAG	GTC	GAT	CAG	ACC	ATG	CTC	GAG	AGT	ACC	TTA	GAA	1440
Thr	Ala	Thr	Phe	Glu	Val	Asp	Gln	Thr	Met	Leu	Glu	Ser	Thr	Leu	Glu	
1105						1110						1115				
GCG	GAC	GTA	TTA	TTT	AAC	TGT	ACC	ACA	TCC	GAG	GGG	AGC	GCA	GGT	CGC	1488
Ala	Asp	Val	Leu	Phe	Asn	Cys	Thr	Thr	Ser	Glu	Gly	Ser	Ala	Gly	Arg	
1120						1125						1130				
GGA	GTC	CTT	GGT	CCA	TTC	GGA	CTT	GTC	GTC	TTA	GCG	GAC	GCA	GAA	AGA	1536
Gly	Val	Leu	Gly	Pro	Phe	Gly	Leu	Val	Val	Leu	Ala	Asp	Ala	Glu	Arg	
1135						1140						1145				
AGC	GAG	CAG	TTG	CCC	GTC	TAT	TTT	TAC	ATT	GCC	AAG	GAC	ACC	GAC	GGT	1584
Ser	Glu	Gln	Leu	Pro	Val	Tyr	Phe	Tyr	Ile	Ala	Lys	Asp	Thr	Asp	Gly	
1150						1155						1160				1165
TCC	AGC	AAG	ACA	TAC	TTC	TGC	GCA	GAT	GAG	TCC	CGC	AGC	AGC	AAC	GAC	1632
Ser	Ser	Lys	Thr	Tyr	Phe	Cys	Ala	Asp	Glu	Ser	Arg	Ser	Ser	Asn	Asp	
1170						1175						1180				
GTC	GAT	ATC	GGC	AAG	TGG	GTC	TAT	GGT	TCG	TCA	GTC	CCA	GTG	TTG	GAG	1680
Val	Asp	Ile	Gly	Lys	Trp	Val	Tyr	Gly	Ser	Ser	Val	Pro	Val	Leu	Glu	
1185						1190						1195				
GGA	GAG	AAA	TTT	AAC	ATG	CGC	CTG	CTT	GTC	GAC	CAC	AGC	ATC	GTC	GAA	1728
Gly	Glu	Lys	Phe	Asn	Met	Arg	Leu	Leu	Val	Asp	His	Ser	Ile	Val	Glu	
1200						1205						1210				
GGC	TTC	GCT	CAG	GGT	GGC	CGT	ACT	GTC	GTA	ACC	AGT	CGT	GTC	TAC	CCT	1776
Gly	Phe	Ala	Gln	Gly	Gly	Arg	Thr	Val	Val	Thr	Ser	Arg	Val	Tyr	Pro	
1215						1220						1225				
GCT	AAA	GCC	ATA	TAT	GGG	GCA	GCC	AAA	CTC	TTC	CTC	TTT	AAT	AAT	GCC	1824
Ala	Lys	Ala	Ile	Tyr	Gly	Ala	Ala	Lys	Leu	Phe	Leu	Phe	Asn	Asn	Ala	
1230						1235						1240				1245
ACA	GGC	ATA	TCA	GTC	AAA	GCC	AGC	TTA	AAA	ATT	TGG	AAA	ATG	AAA	GAG	1872
Thr	Gly	Ile	Ser	Val	Lys	Ala	Ser	Leu	Lys	Ile	Trp	Lys	Met	Lys	Glu	
1250						1255						1260				

GCT CAG TTG GAC CCG TTT CCA TTA AGC GGC TGG TCT AGC
 Ala Gln Leu Asp Pro Phe Pro Leu Ser Gly Trp Ser Ser
 1265 1270

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 637 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala Ser Ser Thr Thr Thr Pro Leu Leu Pro His His His Leu Gln
 1 5 10 15
 Asn Pro Gln Gln Leu Ala Gly Ser Pro Ala Ala His Arg Leu Ser Arg
 20 25 30
 Pro Thr Leu Leu Ser Gly Ile Leu Val Ser Val Leu Val Ile Cys Ala
 35 40 45
 Leu Val Ala Val Ile His Asn Gln Ser Gln Gln Pro Tyr His Asp Gly
 50 55 60
 Gly Ala Lys Pro Ser Ser Ser Ala Ala Thr Thr Thr Phe Pro Thr Ala
 65 70 75 80
 Ser Pro Glu Ala Gly Leu Lys Arg Phe Pro Ile Glu Leu Lys Thr Asn
 85 90 95
 Ala Glu Val Glu Trp Gln Arg Ser Ala Tyr His Phe Gln Pro Asp Lys
 100 105 110
 Asn Tyr Ile Ser Asp Pro Asp Gly Pro Met Tyr His Met Gly Trp Tyr
 115 120 125
 His Leu Phe Tyr Gln Tyr Asn Pro Glu Ser Ala Ile Trp Gly Asn Ile
 130 135 140
 Thr Trp Gly His Ser Val Ser Lys Asp Met Ile Asn Trp Phe His Leu
 145 150 155 160
 Pro Phe Ala Met Val Pro Asp Gln Trp Tyr Asp Ile Glu Gly Val Met
 165 170 175
 Thr Gly Ser Ala Thr Val Leu Pro Asp Gly Gln Ile Ile Met Leu Tyr
 180 185 190
 Thr Gly Asn Ala Tyr Asp Leu Ser Gln Leu Gln Cys Leu Ala Tyr Ala
 195 200 205
 Val Asn Ser Ser Asp Pro Leu Leu Leu Asp Trp Lys Lys Tyr Glu Gly
 210 215 220

Asn Pro Ile Leu Phe Pro Pro Pro Gly Val Gly Tyr Lys Asp Phe Arg
 225 230 235 240
 Asp Pro Ser Thr Leu Trp Leu Gly Pro Asp Gly Glu Tyr Arg Met Val
 245 250 255
 Met Gly Ser Lys His Asn Glu Thr Ile Gly Cys Ala Leu Ile Tyr His
 260 265 270
 Thr Thr Asn Phe Thr His Phe Glu Leu Lys Glu Glu Val Leu His Ala
 275 280 285
 Val Pro His Thr Gly Met Trp Glu Cys Val Asp Leu Tyr Pro Val Ser
 290 295 300
 Thr Thr His Thr Asn Gly Leu Asp Met Val Asp Asn Gly Pro Asn Val
 305 310 315 320
 Lys His Val Leu Lys Gln Ser Gly Asp Glu Asp Arg His Asp Trp Tyr
 325 330 335
 Ala Leu Gly Thr Tyr Asp Val Val Asn Asp Lys Trp Tyr Pro Asp Asp
 340 345 350
 Pro Glu Asn Asp Val Gly Ile Gly Leu Arg Tyr Asp Phe Gly Lys Phe
 355 360 365
 Tyr Ala Ser Lys Thr Phe Tyr Asp Gln His Lys Lys Arg Arg Val Leu
 370 375 380
 Trp Gly Tyr Val Gly Glu Thr Asp Pro Pro Lys Tyr Asp Val Tyr Lys
 385 390 395 400
 Gly Trp Ala Asn Ile Leu Asn Ile Pro Arg Thr Ile Val Leu Asp Thr
 405 410 415
 Lys Thr Asn Thr Asn Leu Ile Gln Trp Pro Ile Ala Glu Val Glu Asn
 420 425 430
 Leu Arg Ser Asn Lys Tyr Asn Glu Phe Lys Asp Val Glu Leu Lys Pro
 435 440 445
 Gly Ser Leu Ile Pro Leu Glu Ile Gly Thr Ala Thr Gln Leu Asp Ile
 450 455 460
 Thr Ala Thr Phe Glu Val Asp Gln Thr Met Leu Glu Ser Thr Leu Glu
 465 470 475 480
 Ala Asp Val Leu Phe Asn Cys Thr Thr Ser Glu Gly Ser Ala Gly Arg
 485 490 495
 Gly Val Leu Gly Pro Phe Gly Leu Val Val Leu Ala Asp Ala Glu Arg
 500 505 510
 Ser Glu Gln Leu Pro Val Tyr Phe Tyr Ile Ala Lys Asp Thr Asp Gly
 515 520 525
 Ser Ser Lys Thr Tyr Phe Cys Ala Asp Glu Ser Arg Ser Ser Asn Asp
 530 535 540

Val Asp Ile Gly Lys Trp Val Tyr Gly Ser Ser Val Pro Val Leu Glu
545 550 555 560

Gly Glu Lys Phe Asn Met Arg Leu Leu Val Asp His Ser Ile Val Glu
565 570 575

Gly Phe Ala Gln Gly Gly Arg Thr Val Val Thr Ser Arg Val Tyr Pro
580 585 590

Ala Lys Ala Ile Tyr Gly Ala Ala Lys Leu Phe Leu Phe Asn Asn Ala
595 600 605

Thr Gly Ile Ser Val Lys Ala Ser Leu Lys Ile Trp Lys Met Lys Glu
610 615 620

Ala Gln Leu Asp Pro Phe Pro Leu Ser Gly Trp Ser Ser
625 630 635

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CLAIMS

1. Nucleic acid molecule encoding a sucrose dependent sucrose fructosyltransferase (SST), selected from the group consisting of
 - (a) nucleic acid molecules encoding a protein comprising the amino acid sequence depicted in SEQ ID No. 2 and SEQ ID No. 4;
 - (b) nucleic acid molecules comprising the nucleotide sequence depicted in SEQ ID No. 1 or a corresponding ribonucleotide sequence;
 - (c) nucleic acid molecules comprising the nucleotide sequence depicted in SEQ ID No. 3 or a corresponding ribonucleotide sequence; and
 - (d) nucleic acid molecules containing a fragment of the nucleic acid molecules mentioned in (a) to (c) encoding a protein that is able to catalyze the linking of β -2,1-glycosidic or β -2,6-glycosidic bonds between fructose units.
2. The nucleic acid molecule according to claim 1, which is a DNA molecule.
3. The DNA molecule according to claim 2, which is a cDNA molecule.
4. The nucleic acid molecule according to claim 1, which is an RNA molecule.
5. Vector containing a nucleic acid molecule according to any one of claims 1 to 4.
6. The vector according to claim 5, wherein the nucleic acid molecule is operatively linked to regulatory elements allowing the transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic cells.
7. The vector according to claim 6, wherein the regulatory elements are derived from the patatin B33 promoter.
8. Host cell transformed with a nucleic acid molecule according to any one of claims 1 to 4 or a vector according to claim 6 or 7 or is derived from such a cell.

9. Method for the production of an SST, wherein the host cell according to claim 8 is cultivated under conditions allowing the synthesis of the SST and the SST is isolated from the cultivated cells and/or the culture medium.
10. SST encoded by a nucleic acid molecule according to any one of claims 1 to 4 or produced according to the method of claim 9.
11. Transgenic plant cell transformed with a nucleic acid molecule according to any one of claims 1 to 4 or a vector according to claim 6 or 7 or derived from such a cell, wherein the nucleic acid molecule encoding an SST from artichoke is controlled by regulatory elements allowing the transcription of a translatable mRNA in plant cells.
12. Plant containing the plant cells according to claim 11.
13. The plant according to claim 12, which is a useful plant.
14. The plant according to claim 13, which is a sucrose or starch-storing plant.
15. The plant according to claim 14, which is a potato plant.
16. Propagation material of a plant according to any one of claims 12 to 15, containing plant cells according to claim 11.
17. Harvest products of a plant of any one of claims 12 to 15, containing plant cells of claim 11.
18. Method for the production of short-chain fructosyl polymers comprising:
 - (a) cultivation of a host cell according to claim 8 or plant cell according to claim 11 under conditions allowing the production of SST and conversion of, if necessary, externally added sucrose or of an equivalent substrate to short-chain fructosyl polymers; and

- (b) obtaining the fructosyl polymers produced this way from the cultivated cells or from the medium.
19. Method for the production of short-chain fructosyl polymers comprising:
- (a) contacting sucrose or an equivalent substrate with an SST according to claim 10 under conditions allowing the conversion to short-chain fructosyl polymers; and
- (b) obtaining the fructosyl polymers so produced.
20. Method for the production of short-chain fructosyl polymers comprising:
- (a) cultivating a plant according to any one of claims 12 to 15; and
- (b) obtaining the fructosyl polymers from these plants or their propagation material according to claim 16 or the harvest products according to claim 17.
21. Use of fructosyl polymers obtainable from a plant cell according to claim 11, a plant according to any one of claims 12 to 15, propagation material according to claim 16 or from a harvest product according to claim 17 or produced according to the method according to any one of claims 18 to 20 for the production of food.
22. Use according to claim 21, wherein the food is baked goods or pasta.
23. Oligonucleotide specifically hybridizing to any one of the nucleic acid molecules according to any one of claims 1 to 4.

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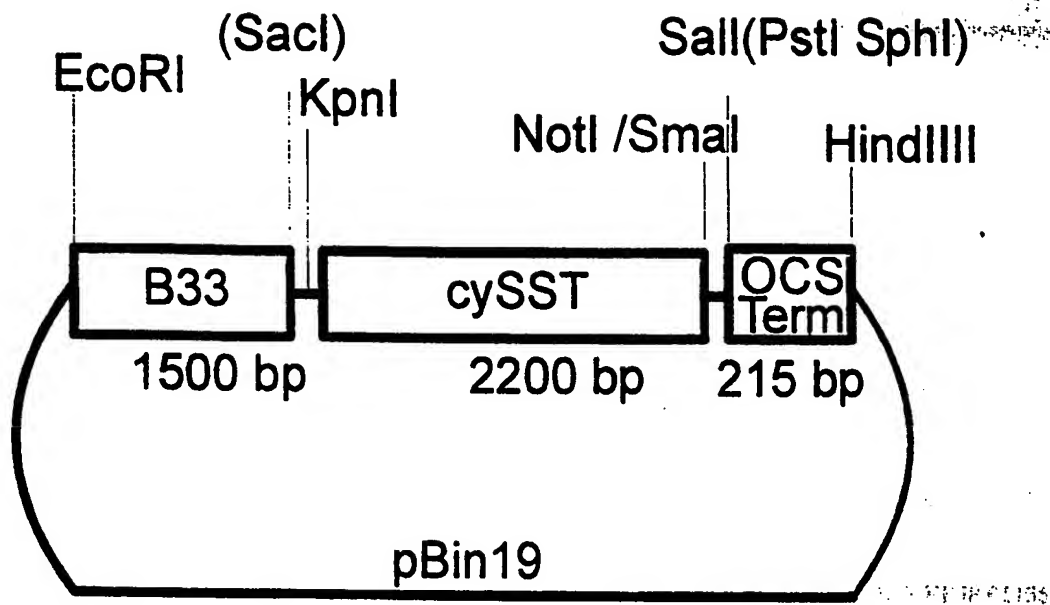


Figure 1

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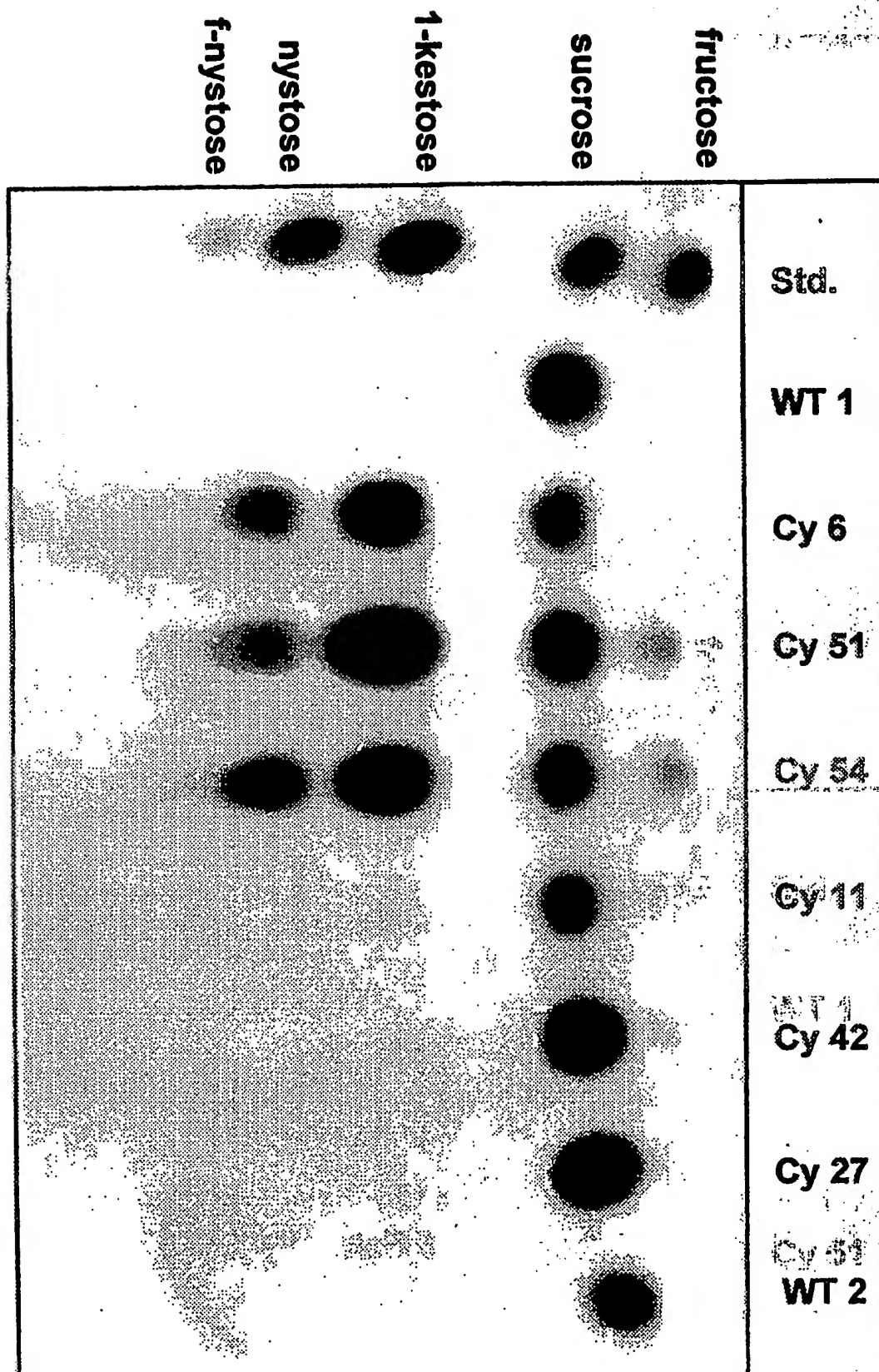


Fig. 2

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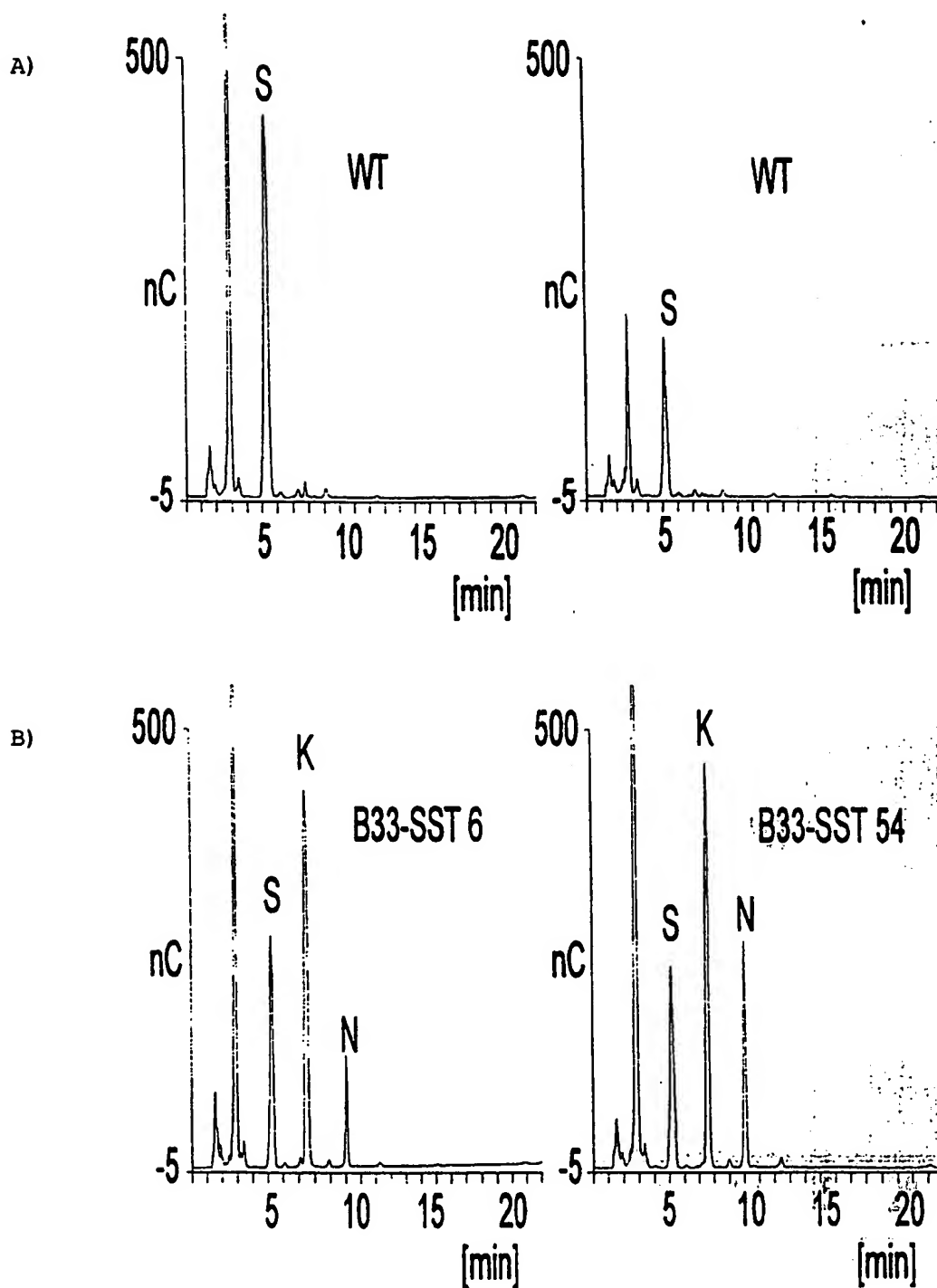


FIGURE 3

SUBSTITUTE SHEET (RULE 26)

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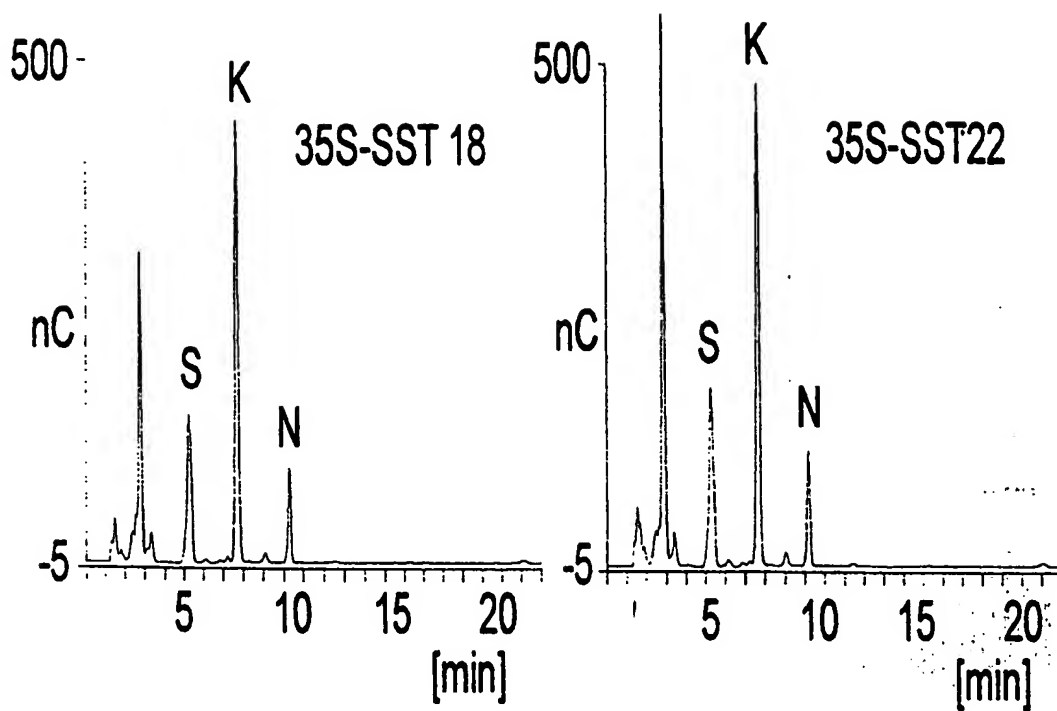


Figure 3 c

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/01156

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N9/10 C12Q1/68 C12P19/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q A01H C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 21023 A (CT VOOR PLANTENVEREDELINGS EN ;TUNEN ARJEN JOHANNES VAN (NL); MEER) 11 July 1996 pages 3,5,6,7,8, page 9, line 35-37, pages 18,19,page 20, line 8-11; examples ---	17,21
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 July 1998

Date of mailing of the international search report

29/07/1998

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Fax: (+31-70) 340-3016

Authorized officer

Holtorf, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/01156

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SPRENGER, N., ET AL. : "fructan synthesis in transgenic tobacco and chicory plants expressing barley sucrose:fructan 6-fructosyltransferase" FEBS LETTERS, vol. 400, 6 January 1997, pages 355-358, XP002071418 abstract, page 356. left column, page 358, Figures ---	17,21
A	WO 95 13389 A (DU PONT ;CAIMI PERRY GERARD (US); HERSHEY HOWARD PAUL (US); KERR P) 18 May 1995 pages 5,6,7,9, page 10, line 26-35; pages 32,33; examples ---	1-23
A	WO 94 14970 A (STICHTING SCHEIKUNDIG ONDERZOE ;SMEEKENS JOSEPHUS CHRISTIANUS (NL)) 7 July 1994 examples and claims ---	1-23
A	VIJN, I., ET AL. : "fructan of the inulin neoseries is synthesized in transgenic chicory plants (Chicorium intybus L.) harbouring onion (Allium cepa L.) fructan:fructan 6G-fructosyltransferase" THE PLANT JOURNAL, vol. 11, no. 3, 3 March 1997, pages 387-398, XP002071419 see the whole document ---	17 Relevant to claim No.
P,X	HELLWEGE, E.M., ET AL. : "transgenic potato tubers accumulate high levels of 1-kestose and nystose: functional identification of a sucrose sucrose 1-fructosyltransferase of artichoke (Cynara scolymus) blossom disc" THE PLANT JOURNAL, vol. 12, no. 5, 5 November 1997, pages 1057-1065, XP002071420 see the whole document ---	1-3, 5-20,23 1-23
P,X	WO 97 29186 A (HAVE D J VAN DER BV ;TURK STEFANUS CORNELIS HENDRIK (NL); GERRITS) 14 August 1997 page 2 , line 28-37; page 3,4,7, examples, claims ---	17,21,23 17
	-/--	

INTERNATIONAL SEARCH REPORT

In International Application No

PCT/EP 98/01156

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>HELLWEGE, E.M., ET AL.: "differences in chain length distribution of inulin from Cynaria scolymus and Helianthus tuberosus are reflected in a transient plant expression system using the respective 1-FFT cDNAs"</p> <p>FEBS LETTERS, vol. 427, 1 May 1998, pages 25-28, XP002071421 see the whole document</p>	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/01156

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9414970 A	07-07-1994	AU 5843794 A HU 71782 A JP 8507918 T NL 9300646 A PL 309606 A EP 0677112 A	19-07-1994 28-02-1996 27-08-1996 18-07-1994 30-10-1995 18-10-1995
WO 9729186 A	14-08-1997	NL 1002275 C AU 1674597 A	08-08-1997 28-08-1997